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Pezicula endophytica sp. nov., endophytic in *Dendrobium* in Thailand

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Abstract—A new species, *Pezicula endophytica,* was isolated from roots and stems of two *Dendrobium* species in northern Thailand. Evidence to support the new species is based on morphology and phylogenetic analysis of the combined ITS, LSU, and RPB2 DNA sequence dataset. *Pezicula endophytica,* which constituted a clade independent from other *Pezicula* species, has 4% distinct base pair differences in all genes. *Pezicula endophytica* has larger macroconidia and longer conidiophores compared with phylogenetically neighboring species. This is the first report of an endophytic *Pezicula* species from *Dendrobium* in Thailand.

Key words— ascomycetes, *Cryptosporiopsis*, *Dermateaceae*, multi-loci, *Orchidaceae*

Introduction

Fungal endophytes in orchids may be symbionts that help plant growth and are possible alternative sources for the bioactive compounds produced in plants (Bungtongdee & al. 2019, Ma & al. 2015, Vujanovic & al. 2000). They also play a role as mycorrhizae in orchids and transfer water and nutrition to the host (Bungtongdee & al. 2019, Ma & al. 2015, Smith & Read 2010). *Pezicula* Tul. & C. Tul. (*Dermateaceae*; anamorph: *Cryptosporiopsis* Bubák & Kabát) is apothecial (Ekanayaka & al. 2017, 2019). The name *Pezicula* has been proposed as the accepted name because *Cryptosporiopsis* species are the asexual morphs of both *Pezicula* and *Neofabraea* H.S. Jacks. (Chen & al. 2016; Johnston & al. 2014; Verkley 1999; Verkley & al. 2003; Wijayawardene & al. 2017a,b). *Pezicula* is characterized by brightly colored, short-stalked apothecia with circular discs that are initially concave and surrounded by slightly elevated margins but later become convex. They occur on the bark of woody plants and are involved in wood degradation (Kowalski 1996). Asci are inoperculate and mostly clavate or cylindric-clavate, ascospores vary in shape from broadly ellipsoid to elongate-ellipsoid to allantoid or fusoid (Ekanayaka & al. 2016, Ooki & al. 2003, Verkley 1999, Verkley & al. 2003). The asexual morph of *Pezicula* species produces sporodochial conidiomata, with macro- and microconidia formed on different conidiophores (Ekanayaka & al. 2016, Verkley 1999). *Pezicula* species are recorded as endophytes, plant pathogens, and saprobes from a wide range of hosts (Chen & al. 2016, Wijayawardene & al. 2017a). They have been isolated as endophytes from *Dendrobium nobile* Lindl. and *Holcoglossum wangii* Christenson (*Orchidaceae*) in China (Chen & al. 2011, Tan & al. 2012).

Dendrobium Sw. is one of the largest genera in *Orchidaceae* (https://www.kew.org/), and many species are important medicinal and ornamental plants (Ma & al. 2015). However, few reports concern endophytic *Pezicula* species in *Dendrobium* (Chen & al. 2011, Tan & al. 2012).

In this study, we isolated four endophytic fungal strains from two different, undetermined *Dendrobium* species sampled in northern Thailand. The strains were identified as representing a new species, *Pezicula endophytica,* based on morphological and phylogenetic analyses. This is the first report of an endophytic *Pezicula* in *Dendrobium* in Thailand.

Materials & methods

Sample collection

Healthy roots and stems of two different but undetermined *Dendrobium* species (sp.1 and sp.2) were collected from outside the temple of Wat Phra That Doi Tung (Temple of Doi Tung Pagoda), Mae Fah Luang district, Chiang Rai Province, Thailand. Fresh material was kept fresh by placing into Zip-lock bags or tubes containing silica gel on ice.

Fungal isolation & cultivation

The material was processed within 48 hours. Surface sterilization followed Nontachaiyapoom & al. (2010) with minor modifications. Healthy roots and stems were washed in tap water and then immersed for 5 minutes in a solution containing 3% (v/v) H_2O_2 and 70% (v/v) ethanol, and then rinsed with three changes of sterile distilled water. The sterilized materials were cut into 2 mm^2 pieces and placed on potato dextrose agar (PDA, Difco, BBL/USA' #213400) containing 50 µg/ml oxytetracycline, 50 µg/ml penicillin, and 50 µg/ml streptomycin (Otero & al. 2002). The surface sterilization method was checked, as described by Petrini (1991). Petri dishes were incubated at 28 °C under natural light and observed daily; mycelia on the edge of developing fungal colonies were transferred to fresh PDA to obtain pure cultures. The pure cultures were deposited in Mae Fah Luang University Culture Collection, Chiang Rai, Thailand (MFLUCC). Dry cultures of the new species were deposited in the herbarium of Mae Fah Luang University, Chiang Rai, Thailand (MFLU).

Morphological analysis

Pure cultures were cultivated on both PDA and water agar (WA) media in a dark cabinet at room temperature (28 °C) and observed every week. Sterilized toothpicks were placed on the WA surface to encourage fungal sporulation. The growth rate was evaluated after mycelia had nearly covered the whole agar surface. Any observable structures were examined using a Zeiss SteREO Discovery.V8 stereomicroscope. The colonies were examined for morphological characteristics and structures were removed and mounted in water. The mounted slides were observed using a Nikon EOS 700D compound microscope. All observed structures were measured with Image Frame Work v.0.9.7 and the photo plates were assembled using Photoshop CS 6.0.

DNA extraction & amplification

Genomic DNA was extracted from mycelium of endophytic fungi using an EZgene Fungal gDNA Kit following the manufacturer's instructions. The selected genes for amplification were partial nuclear rDNA internal transcribed spacer (ITS), partial large subunit (LSU), and partial RNA polymerase II large subunit 2 (RPB2). Each 25 µl amplification reaction contained 12.5 µl of 2*Bench TopTM Taq Master Mix (0.05 units/µl Taq DNA polymerase, 0.4 mM dNTPs and 4 mM $MgCl_2$); 2 µl forward and reverse primers; 1 µl of DNA template and 9.5 µl of threefold-distilled water. The primers and PCR protocol followed Chen & al. (2016). PCR products were visualized on 1% agarose gel stained with Realtimes Biotech Goldview and then sent to Sangon Biotech for purification and sequencing. All DNA sequences from this study were submitted to GenBank.

TABLE. 1. Fungal strains and sequences of *Pezicula* and related taxa sourced from the present study, BLAST searches in GenBank, or Chen & al. (2016).

SPECIES	STRAIN	GENBANK ACCESSION NO.		
		ITS	LSU	RPB2
Neofabraea.inaequalis	**CBS 326.75**	KR859081	KR858872	KR859321
N. krawtzewii	CBS 102867	KR859084	KR858875	KR859324
N. malicorticis	CBS 102863	KR859085	KR858876	KR859325
	CBS 122030	KR859086	KR858877	KR859326
Parafabraea caliginosa	**CBS 124806**	KR859090	KR858881	KR859330
Pa. eucalypti	**CBS 124810**	KR859091	KR858882	KR859331
Pezicula acericola	CBS 239.97	KR859093	KR858884	KF376214
	CBS 245.97	KR859098	KR858889	KF376213
P. aurantiaca	CBS 201.46	KR859102	KR858893	KF376210
P. brunnea	**CBS 120291**	KR859103	KR858894	—
P. californiae	**CBS 124805**	KR859104	KR858895	KR859332
P. carpinea	**CBS 923.96**	KR859108	KR858899	KF376158
	CBS 921.96	KR859107	KR858898	KF376159
P. chiangraiensis	**MFLUCC 15-0170**	KU310621	KU310622	KU310623
P. cinnamomea	CBS 626.96	KR859152	KR858944	KF376162
	CBS 239.96	KR859124	KR858915	KF376165
P. cornina	CBS 285.39	KR859163	KR858955	KR859333
P. corticola	CBS 259.31	KR859164	KR858956	—
	CBS 260.31	KR859165	KR858957	—
P. corylina	CBS 140.22	KR859166	KR858958	KR859334
	CBS 249.97	KR859168	KR858960	KF376161
P. diversispora	**CBS 185.50**	KR859170	KR858962	—
	CBS 282.47	KR859171	KR858963	—
P. endophytica	**MFLUCC 14-0116**	MN908669	MN908642	MT371880
	MFLUCC 14-0118	MN908666	MN908640	—
	MFLUCC 14-0140	MN908667	MN908641	—
	MFLUCC 14-0144	MN908668	—	—
P. ericae	**CBS 120290**	KR859173	KR858965	—
	CBS 120292	KR859174	KR858966	—
P. eucrita	CBS 259.97	KR859179	KR858971	KF376205
	CBS 656.96	KR859185	KR858977	KF376208
P. fagacearum	**CBS 112400**	KR859201	KR858993	KR859335
	CBS 112402	KR859203	KR858995	KR859336
P. frangulae subsp. *frangulae*	CBS 100244	KR859204	KR858996	KF376211
	CBS 778.96	KR859209	KR859001	KF376212
P. heterochroma	**CBS 199.46**	KR859210	KR859002	—
P. melanigena	**CBS 898.97**	KR859211	KR859003	—
P. microspora	**CBS 124641**	KR859212	KR859004	KR859337
P. neocinnamomea	**CBS 100248**	KR859213	KR859005	KF376209
P. neoheterochroma	**CBS 127388**	KR859221	KR859013	KR859338

Species	Strain	GenBank accession No.		
		ITS	LSU	RPB2
P. neosporulosa	**CBS 101.96**	KR859223	KR859015	KF376193
	CBS 102.96	KR859224	KR859016	KF376181
P. ocellata	CBS 268.39	KR859232	KR859024	KR859339
	CBS 949.97	KR859233	KR859025	KF376215
P. pruinosa	CBS 292.39	KR859234	KR859026	—
P. pseudocinnamomea	**CBS 101000**	KR859235	KR859027	KR859340
P. querciphila	**CBS 134525**	JX144750	—	—
P. radicicola	**CBS 640.94**	KR859236	KR859028	—
	CBS 681.83	KR859237	KR859029	—
P. rubi	CBS 253.97	KR859250	KR859042	KF376204
	CBS 593.96	KR859253	KR859045	KF376203
P. sporulosa	**CBS 224.96**	KR859261	KR859053	KF376201
	CBS 225.96	KR859262	KR859054	KF376202
Phlyctema vagabunda	CBS 109875	KR859275	KR859069	KR859346
	CBS 304. 62	KR859276	KR859070	KR859347
Rhizodermea veluwensis	**CBS 110605**	KR859282	KR859076	KR859353
	CBS 110615	KR859283	KR859077	KR859354

*Ex-type, ex-epitype or other type strains are in **bold**

Sequence analysis

The sequencing results were assembled using DNASTAR SeqMan v.7.1.0. The assembled sequences were subjected to nucleotide BLAST searches in GenBank (www.ncbi.nlm.nih.gov/BLAST) for establishing highly similar strains (Altschul & al. 1997). Supplemental strains of *Pezicula* and related genera were selected from Chen & al. 2016, especially ex-type and ex-epitype sequences where available. Sequence alignment was carried out by MAFFT v.7.0 (https://mafft.cbrc.jp/alignment/server/). Misaligned sequences were manually edited using AliView v.1.26 (Larsson 2014). The three loci were combined into a concatenated dataset with SequenceMatrixa 1.7.8 (Vaidya & al. 2011).

The best fitting nucleotide substitution models for each gene were estimated by IQ-TREE (Nguyen & al. 2014) with ultrafast bootstrap (Hoang & al. 2017). Gaps were regarded as missing data. Both maximum likelihood (ML) and Bayesian (BI) trees were constructed on The CIPRES Science Gateway V. 3.3 (http://www.phylo.org/index.php/). The ML tree was constructed using RAxML-HPC2 on XSEDE. Maximum likelihood bootstrap values (MLSB) were calculated with 1000 iterations. The generalized time-reversible (GTR) was set with gamma distribution. The BI tree was implemented by MrBayes on XSEDE (3.2.6). Two sets of four simultaneous independent chains of Markov chains Monte Carlo (MCMC) simulations were run for 5,000,000 generations; 25% of trees were discarded and the remaining trees were used to calculate the Bayesian posterior probabilities (PP). Convergence was assumed when the standard deviation of split sequences was less than 0.01. The generated trees were submitted to TreeBase (http://purl.org/phylo/treebase/phylows/study/TB2:S25576). The resulting trees were viewed with Figtree v.1.4.0. The layout was made with Adobe Illustrator CS 6.

Results

Fungal isolation & sporulation

Four endophytic *Pezicula* strains were isolated from healthy roots and stems of *Dendrobium* species. Isolate MFLUCC 14-0116 sporulated on PDA after one month and on WA with toothpicks after two months. The conidia, conidiomata, conidiophores and mycelia were recorded from cultures.

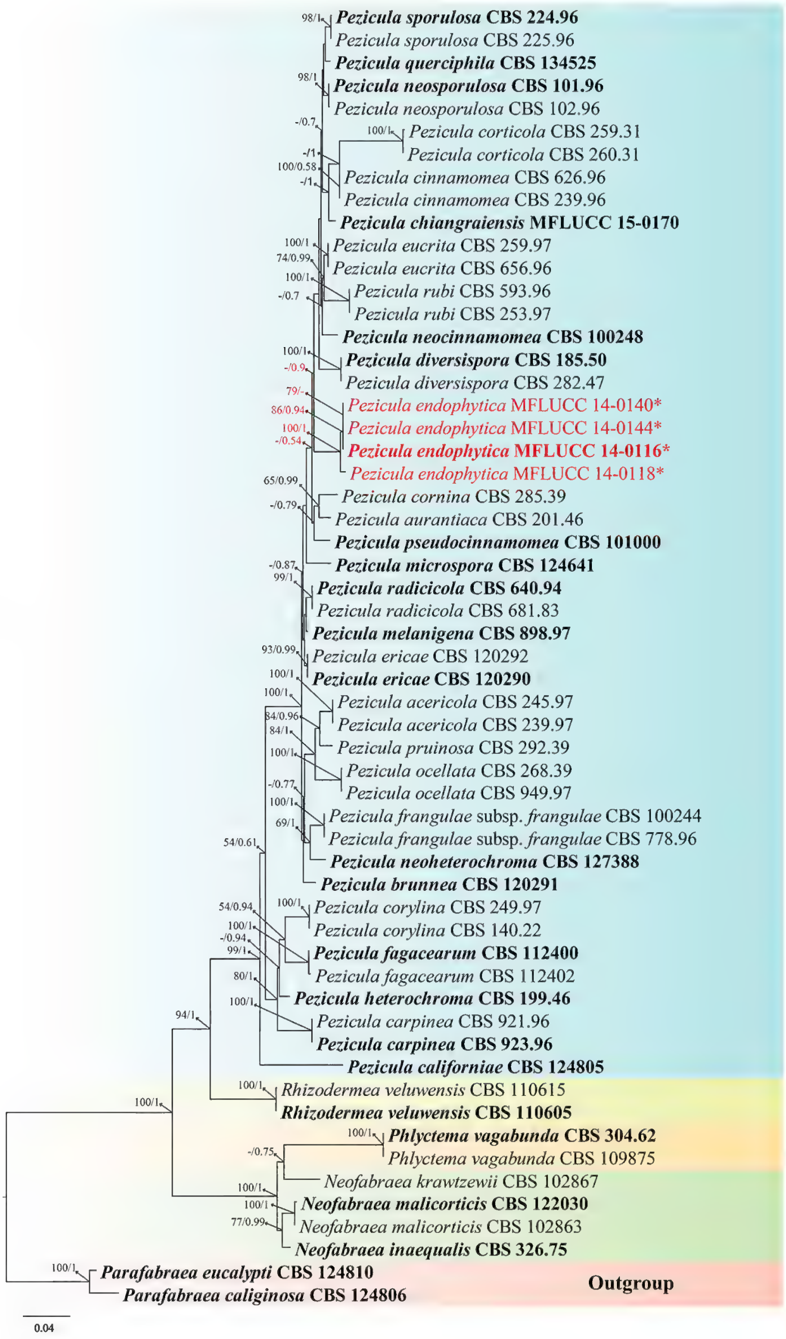
Phylogenetic results

Sequences of the isolated *Pezicula* and reference sequences of related taxa are listed in Table 1. Combined data for the phylogenetic tree comprised 57 sequences containing 2478 sites (including 582 ITS sites, 843 LSU sites, and 1053 RPB2 sites). The selected nucleotide substitutional model for ITS and RPB2 was a symmetrical model (SYM) with gamma distribution, and for LSU was Kimura's two-parameter substitution model with invgamma distribution.

The phylogenetic tree (Fig. 1) was rooted with two selected outgroup species *Parafabraea caliginosa* (CBS 124806) and *P. eucalypti* (CBS 124810) with robust bootstrap support (ML = 100%, PP = 1). In the phylogenetic tree (RAxML), *Pezicula endophytica* strains clustered and formed an independent clade. Strains MFLUCC 14-0140, MFLUCC 14-0144 and MFLUCC 14-0116 clustered with MFLUCC 14-0118 (ML = 100%, PP = 1). *Pezicula aurantiaca*, *P. cornina* and *P. pseudocinnamomea* formed a clade close to *P. endophytica*. The neighboring genera *Neofabraea* H.S. Jacks., *Phlyctema* Desm. and *Rhizodermea* Verkley & Zijlstra were well separated from *Pezicula* (ML = 94%, PP = 1).

Pezicula endophytica (MFLUCC 14-0116, ex-type) differs from *Pezicula diversispora* (CBS 185.50, ex-type) in 4% of base pairs [2.9% in ITS (17/582bp), 1.1% in LSU (9/843bp)] and from *Pezicula cornina* (CBS 285.39) in 8.6% of base pairs [3.3% in ITS (19/582bp); 1.5% in LSU (13/843bp); 3.8% in RPB2 (40/1053bp)].

Fig. 1. The consensus phylogram resulting from a RAxML analysis of the combined three loci (ITS-LSU-RPB2) of *Pezicula* and related taxa. *Parafabraea caliginosa* and *P. eucalypti* were selected as outgroup taxa. Isolates generated in this study are labelled with *. Holotype and ex-type isolates are in bold. Support values from maximum likelihood (MLBS) and Bayesian posterior probabilities (PP) are labelled at the end of nodes (50% majority rule). Dashes indicate values ≤50%. Scale bar represents 0.04 substitutions per site.


98/1
Pezicula sporulosa CBS 224.96
Pezicula sporulosa CBS 225.96
Pezicula querciphila CBS 134525
98/1
Pezicula neosporulosa CBS 101.96
Pezicula neosporulosa CBS 102.96
-/0.7
100/1
Pezicula corticola CBS 259.31
Pezicula corticola CBS 260.31
-/1
100/0.58
-/1
Pezicula cinnamomea CBS 626.96
Pezicula cinnamomea CBS 239.96
Pezicula chiangraiensis MFLUCC 15-0170
100/1
74/0.99
Pezicula eucrita CBS 259.97
Pezicula eucrita CBS 656.96
100/1
-/0.7
Pezicula rubi CBS 593.96
Pezicula rubi CBS 253.97
Pezicula neocinnamomea CBS 100248
100/1
-/0.9
Pezicula diversispora CBS 185.50
Pezicula diversispora CBS 282.47
79/-
86/0.94
100/1
-/0.54
Pezicula endophytica MFLUCC 14-0140*
Pezicula endophytica MFLUCC 14-0144*
Pezicula endophytica MFLUCC 14-0116*
Pezicula endophytica MFLUCC 14-0118*
65/0.99
-/0.79
Pezicula cornina CBS 285.39
Pezicula aurantiaca CBS 201.46
Pezicula pseudocinnamomea CBS 101000
Pezicula microspora CBS 124641
-/0.87
99/1
Pezicula radicicola CBS 640.94
Pezicula radicicola CBS 681.83
Pezicula melanigena CBS 898.97
93/0.99
Pezicula ericae CBS 120292
Pezicula ericae CBS 120290
100/1
100/1
84/0.96
84/1
Pezicula acericola CBS 245.97
Pezicula acericola CBS 239.97
Pezicula pruinosa CBS 292.39
100/1
Pezicula ocellata CBS 268.39
Pezicula ocellata CBS 949.97
-/0.77
100/1
69/1
Pezicula frangulae subsp. frangulae CBS 100244
Pezicula frangulae subsp. frangulae CBS 778.96
Pezicula neoheterochroma CBS 127388
54/0.61
Pezicula brunnea CBS 120291
54/0.94
100/1
Pezicula corylina CBS 249.97
Pezicula corylina CBS 140.22
100/1
-/0.94
99/1
Pezicula fagacearum CBS 112400
Pezicula fagacearum CBS 112402
80/1
Pezicula heterochroma CBS 199.46
100/1
94/1
Pezicula carpinea CBS 921.96
Pezicula carpinea CBS 923.96
Pezicula californiae CBS 124805
100/1
Rhizodermea veluwensis CBS 110615
100/1
Rhizodermea veluwensis CBS 110605
100/1
Phlyctema vagabunda CBS 304.62
-/0.75
Phlyctema vagabunda CBS 109875
Neofabraea krawtzewii CBS 102867
100/1
100/1
Neofabraea malicorticis CBS 122030
77/0.99
Neofabraea malicorticis CBS 102863
Neofabraea inaequalis CBS 326.75
100/1
Parafabraea eucalypti CBS 124810
Parafabraea caliginosa CBS 124806
Outgroup
0.04

Taxonomy

Pezicula endophytica X.Y. Ma, K.D. Hyde & J.C. Kang, sp. nov. FIG. 2

IF 558634

Differs from *Pezicula diversispora* by its longer conidiophores and narrower macroconidia.

TYPE—Thailand, Chiang Rai Province, Mae Fah Luang district, outside Temple of Doi Tung Pagoda, endophytic in the roots of *Dendrobium* sp. 1, 19 December 2013, Nontachaiyapoom S, Aewsakul N & Ma XY (**holotype**, MFLU 17-2789; ex-type living culture, MFLUCC 14-0116; **isotype**, MFLU 20-0442; ex-type living culture, MFLUCC 14-0118.

ETYMOLOGY—in reference to its endophytic habit.

SEXUAL MORPH undetermined. ASEXUAL MORPH observed on both PDA and (with toothpicks) WA.

COLONIES on WA superficial, white with light brownish grey mycelia, sparse, irregular edge, fluffy, reverse light brown, growth rate 0.8 mm/day. MYCELIUM hyaline to light brown, smooth, 2.5–4.4 µm diam. CONIDIOMATA on toothpicks on WA aggregated or scattered, with aerial and fluffy hyphae, yellow-grey, globose.

COLONIES on PDA medium superficial, white and brown in the center, lobate edge, dense, fluffy, concentric ring close to the edge, reverse brown, growth rate: 1.2 mm/day. CONIDIOMATA erumpent on surface, aggregated or scattered, with irregular sporodochia, with brown to milky white conidial masses, globose to irregular. MACROCONIDIOPHORES (16–)22–30(–40) × (2–)3–4(–5.5) µm (x = 26 × 3.5 µm, n=5), arising from the inner wall layers of conidiomata, hyaline, cylindrical, straight to flexuous, septate, unbranched or branched, smooth-walled. MACROCONIDIOGENOUS CELLS hyaline, enteroblastic, phialidic, determinate, proliferating percurrently, sometimes with a minute collarette, cylindrical, integrated, determinate, smooth-walled. MACROCONIDIA (30–)31–43(–44) × (5–)7–9 (–10) µm (x = 37 × 8 µm, n=16), L/W=4.4, hyaline, elongated ellipsoid, rounded at the apex, narrow and slightly truncate at the base, sometimes attenuated and with a protruding scar at the base, septate when mature, straight, smooth-walled, with guttules.

ADDITIONAL SPECIMENS EXAMINED—**THAILAND, CHIANG RAI PROVINCE, Mae Fah Luang district**, outside Temple of Doi Tung Pagoda, endophytic in roots and stems of *Dendrobium* sp. 2, 19 December 2013, Nontachaiyapoom S, Aewsakul N & Ma XY. (MFLUCC 14-0140, GenBank MN908667, MN908641; MFLUCC 14-0144, GenBank MN908668).

COMMENTS—*Pezicula endophytica* formed an independent clade differing from other species in the phylogenetic tree, and is adjacent to *P. aurantiaca*,

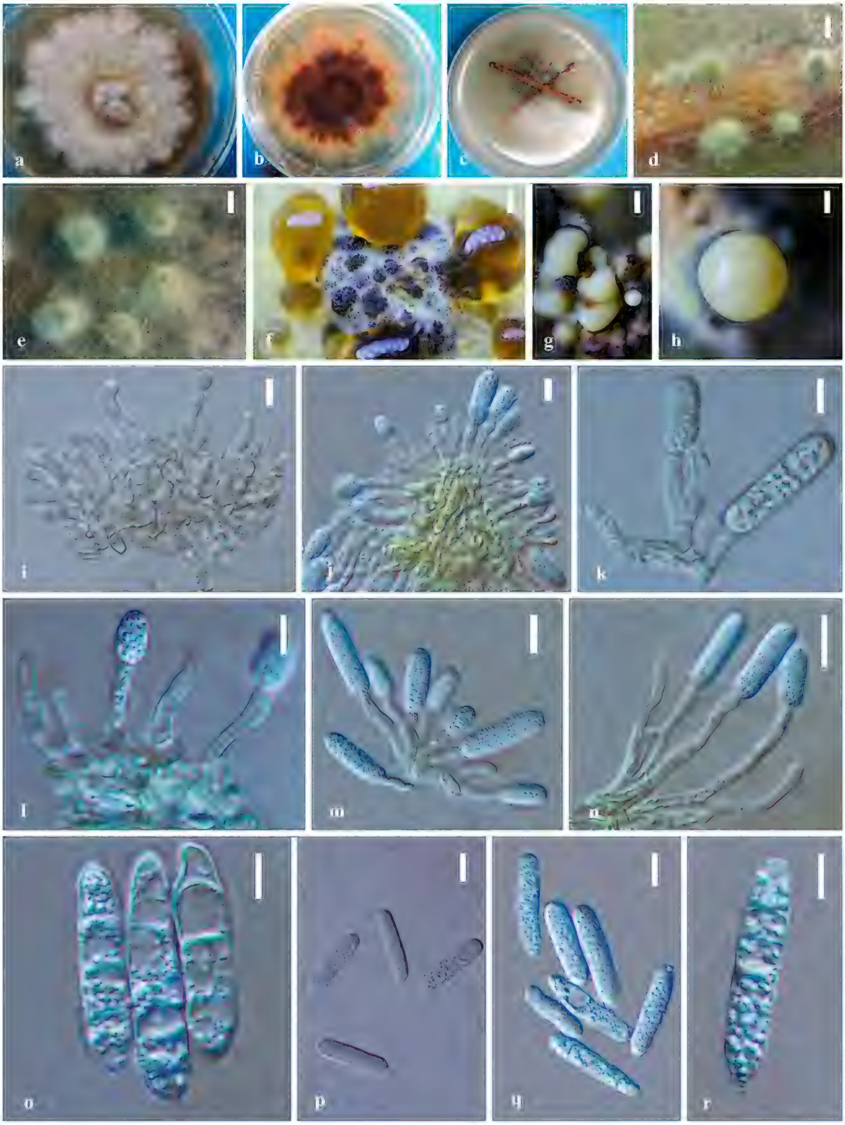


Fig. 2. *Pezicula endophytica* (ex-holotype MFLUCC 14-0116). a, b. Colony on PDA (a. top; b. reverse). c. Colony on WA with toothpicks. d, e. Conidiomata on toothpicks on WA. f–h. Conidiomata with conidial masses on PDA. i–n. Macroconidiophores, macroconidiogenous cells and macroconidia. o–r. Macroconidia. Note: i–r from PDA. Scale bars: d–h = 500 µm; i–n, p, q = 10 µm; o, r = 5 µm.

P. cornina, *P. diversispora,* and *P. pseudocinnamomea*. *Pezicula endophytica* is distinguished from those species by its longer macroconidiophores and narrower macroconidia with septa (*P. aurantiaca* and *P. cornina* are aseptate; Chen & al. 2016, Robak 1950, Verkley 1999). *Pezicula endophytica* (ex-type MFLUCC 14-0116) has >4% different gene sequences than *P. diversispora* (ex-type CBS 185.50). Compared with their cultures on MEA, *Pezicula endophytica* produces no diffusing pigment on WA (Verkley 1999). *Pezicula aurantiaca*, *P. cornina*, *P. diversispora,* and *P. pseudocinnamomea* were isolated from dead branches, bark, and twigs of dicotyledonous woody plants from European countries (Chen & al. 2016, Robak 1950, Verkley 1999), whereas *P. endophytica* is an endophytic fungus from monocotyledonous *Dendrobium* orchids.

Discussion

The taxonomy and identification of *Pezicula* is complicated because many species have been introduced based on single-morph taxa, some lack molecular data, and their morphology may vary depending on the growing medium (Verkley 1999, Verkley & al. 2003). Of the 136 species listed in *Pezicula* (http://www.speciesfungorum.org), only 29 (including *P. endophytica*) have DNA sequence data available in the 2021 NCBI database (Yuan & al. 2015, Ekanayaka & al. 2016; http://www.ncbi.nlm.nih.gov/). A revision of the genus based on comprehensive morphological and phylogenetic data is necessary. The asexual morphs of most *Pezicula* species produce both macro- and microconidia on the natural substrata and in cultures (Verkley 1999). In this study, only macroconidia were observed in cultures. Macroconidia of *P. endophytica* resemble *P. diversispora,* which differs by its longer conidiophores, narrower macroconidia, and a 4% gene sequence difference. *Pezicula endophytica,* which only produced its asexual morph in cultures, differs from *P. carpinea* (the type species) by lacking conidia along the conidiophore sides and large gene sequence differences. Presumably *P. endophytica* microconidia will not be produced on WA after two sporulation months.

Chen & al. (2011) first reported an endophytic *Pezicula* sp. from *Dendrobium* orchids in China. *Pezicula endophytica* was the first *Pezicula* endophyte identified in Thailand. *Pezicula* species are frequently isolated endophytes in angiosperms, particularly in roots of *Abies*, *Calluna*, *Erica*, *Larix*, *Pleurothallis*, *Pseudorchis*, *Stelis,* and *Vaccinium* (Chen & al. 2016, Devi & Joshi 2014, Herrera & al. 2010, Kohout & al. 2013, Noble & al. 1991, Schulz & al. 2002, Strobel & al. 1999, Talontsi & al. 2012, Verkley & al. 2003, Wang & al. 2014, Yuan & Verkley 2015, Zilla & al. 2013). They have also been found in peridermal bark of living branches of temperate trees such as *Abies*, *Acer*, *Alnus*, *Betula*, *Carpinus*, *Castanea*, *Fagus*, *Fraxinus*, *Picea*, *Pinus*, *Quercus*, and *Sequoia* (Espinosa-Garcia & Langenheim 1990, Kowalski & Kehr 1992, Matsumura & al. 2013, Petrini & Müller 1979, Schulz & al. 1995, Sieber 1988, Sieber & Dorworth 1994, Verkley 1999).

Although many *Pezicula* species are reported from temperate and boreal forests of the northern hemisphere, such as Canada, Germany, and the Netherlands (Kowalski & Kehr 1992, Chen & al. 2016, Verkley 1999), few geographic and climatic limitations have been observed. Saprobic and pathogenic *Pezicula* species have been recorded from temperate to tropical and Mediterranean areas (Bergero & al. 2003, Cheewangkoon & al. 2010, Old & al. 2002). *Pezicula eucalyptigena* was isolated from leaves of *Eucalyptus* sp. in the mediterranean Western Cape, South Africa, which has smaller conidia ((23–)24–27(–30) × (6–)7(–8) µm; Crous & al. 2019) than those of *P. endophytica*. Ekanayaka & al. (2016) described a sexual morph from Thailand, *P. chiangraiensis*, saprobic on the bark of decaying wood. *Pezicula ericae* was found as ericoid mycorrhizal fungi in the roots of velvetleaf blueberry (*Vaccinium myrtilloides*) seedlings in Canada, which could enhance development of the host plant (Mu 2020). For most endophytic *Pezicula* species, their roles remain unknown.

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